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Microlunatus parietis sp. nov., isolated from an indoor wall

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A Gram-positive, coccoid, non-endospore-forming actinobacterium (strain 12-Be-011^T) was isolated from indoor wall material. Based on 16S rRNA gene sequence comparisons, strain 12-Be-011^T was clearly shown to belong to the genus *Microlunatus* and was most closely related to *Microlunatus panaciterrae* Gsoil 954^T (95.7 %), *Microlunatus soli* CC-12602^T (94.9 %), *Microlunatus ginsengisoli* Gsoil 633^T (94.8 %), *Microlunatus aurantiacus* YIM 45721^T (95.5 %) and *Microlunatus phosphovorus* DSM 10555^T (94.7 %). The cell-wall peptidoglycan contained LL-diaminopimelic acid as the diagnostic diamino acid. Mycolic acids were absent. The major menaquinone was MK-9(H_4). The polar lipid profile consisted of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown glycolipid. The major fatty acids of iso-$C_{15:0}$, anteiso-$C_{15:0}$ and iso-$C_{16:0}$ supported the affiliation of strain 12-Be-011^T to the genus *Microlunatus*. Physiological and biochemical test results allowed a clear phenotypic differentiation of strain 12-Be-011^T from all other species of the genus *Microlunatus*. Hence, strain 12-Be-011^T can be regarded as a representative of a novel species, for which the name *Microlunatus parietis* sp. nov. is proposed, with the type strain 12-Be-011^T (=DSM 22083^T=CCM 7636^T).

The genus *Microlunatus*, as proposed by Nakamura *et al.* (1995), currently contains five recognized species, *Microlunatus phosphovorus* (Nakamura *et al.*, 1995), *M. ginsengisoli* (Cui *et al.*, 2007), *M. aurantiacus* (Wang *et al.*, 2008), *M. panaciterrae* (An *et al.*, 2008) and *M. soli* (Kämpfer *et al.*, 2010). All these species are characterized chemotaxonomically by the presence of LL-diaminopimelic acid (LL-Dpm) in the cell-wall peptidoglycan, by MK-9(H_4) as the predominant menaquinone and the predominance of anteiso-$C_{15:0}$, iso-$C_{15:0}$ and iso-$C_{16:0}$ as the major fatty acids.

Strain 12-Be-011^T was enriched and recovered from interior wall plaster colonized with moulds in a house in Berlin, Germany, using previously described procedures (Schäfer *et al.*, 2010). The novel strain was maintained on M79 agar (www.dsmz.de) and preserved at −80 °C by mixing well grown M79 broth cultures in a 1:1 ratio with glycerol preservation medium containing K_2HPO_4 (1.26 %), KH_2PO_4 (0.36 %), $MgSO_4.7H_2O$ (0.01 %), $C_6H_5Na_3O_7.2H_2O$ (0.09 %), $(NH_4)_2SO_4$ (0.18 %) and glycerol (8.8 %). Stock cultures of the isolate in liquid M79 supplemented with 5 % DMSO were also maintained in the vapour phase of liquid nitrogen.

Gram-staining behaviour and cell morphology were observed microscopically as described by Kämpfer & Kroppenstedt (2004). Strain 12-Be-011^T formed beige pigmented colonies with a characteristic wrinkly and shiny surface. Cells were Gram-positive, non-motile irregular rods. In older cultures (>5 days of growth), cells changed their shape to short rods and coccoid forms. DNA isolation was performed with a commercial DNA extraction kit (GenElute Plant Genomic DNA mini prep kit, Sigma) after disruption of cells by a 1 min bead-beating step with 1 g 0.1 Ø Zirconia beads. Multiple sequence alignment and analysis of the data were performed with the MEGA (molecular evolutionary genetics analysis) software package version 4 (Tamura *et al.*, 2007) and the ARB software package (December 2007 version; Ludwig *et al.*, 2004) and the corresponding SILVA SSURef 95 database (release July 2008; Pruesse *et al.*, 2007). Phylogenetic trees were constructed with the neighbour-joining method (Fig. 1) and the maximum-likelihood method with fastDNAml (Olsen *et al.*, 1994, results not

Abbreviation: LL-Dpm, LL-diaminopimelic acid.

The GenBank/EMBL/DDBJ accession number for the 16S rRNA gene sequence of strain 12-Be-011^T is FN556016.

A supplementary table detailing fatty acid contents for strain 12-Be-011^T and related species and a supplementary figure showing the results of the polar lipid analysis are available with the online version of this paper.

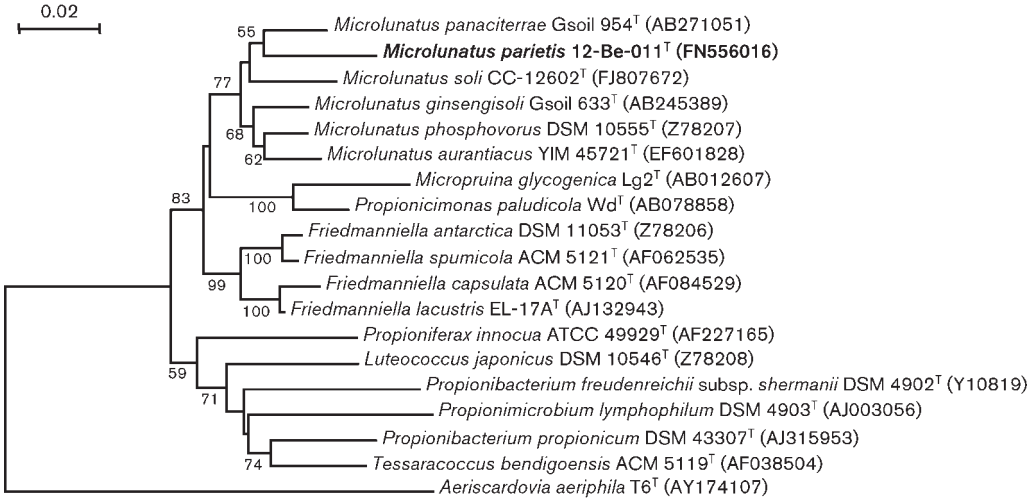

Fig. 1. Phylogenetic analysis based on 16S rRNA gene sequences available from the European Molecular Biology Laboratory database (accession numbers are given in parentheses). Multiple alignment, distance calculations (distance options according to the Kimura-2 model) and clustering with the neighbour-joining method were performed by using the MEGA software package (molecular evolutionary genetics analysis) version 4 (Tamura *et al.*, 2007). Bootstrap values based on 1000 replications are listed as percentages at the branching points. Bar, 0.02 substitutions per nucleotide position.

shown). Bootstrap values were based on 1000 replications. The 16S rRNA gene sequence of strain 12-Be-011^T was a continuous stretch of 1367 bp.

Sequence similarity calculations after pairwise local alignment showed that the closest relatives of strain 12-Be-011^T were *M. panaciterrae* Gsoil 954^T (95.7 %), *M. aurantiacus* YIM 45721^T (95.5 %), *M. soli* CC-12602^T (94.9 %), *M. ginsengisoli* Gsoil 633^T (94.8 %) and *M. phosphovorus* DSM 10555^T (94.7 %).

Bacterial biomass for chemotaxonomic investigations was prepared by cultivating strain 12-Be-011^T for 120 h in shake flasks in liquid organic medium M79 at 180 r.p.m. at 28 °C. For fatty acid analyses, cells were grown on R2A agar (Oxoid) at 28 °C for 5 days.

Cell wall analysis was performed as described previously (Groth *et al.*, 1996). The amino acids and peptides of cell-wall hydrolysates were analysed by TLC on cellulose plates using the solvent systems as described by Schleifer & Kandler (1972). The peptidoglycan hydrolysates contained LL-Dpm as the diagnostic diamino acid. The whole-cell sugars, determined by TLC (Becker *et al.*, 1965), were arabinose, galactose, rhamnose and glucose.

Menaquinones were extracted as described by Collins *et al.* (1977) and analysed by HPLC (Groth *et al.*, 1996). In common with all other species of the genus *Microlunatus*, strain 12-Be-011^T exhibited a quinone system with a high amount of MK-9(H_4) (58 %), followed by MK-8(H_2) (28 %). Minor amounts of MK-8 (10 %) and MK-9 (3 %) were also detected. The absence of mycolic acids was shown by TLC as described by Minnikin *et al.* (1975). Polar lipids were extracted and identified by TLC as described by Minnikin *et al.* (1979). The major lipids were diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown glycolipid (see Supplementary Fig. S1 in IJSEM Online). The same complex pattern of phospholipids with the major components and some unknown glyco- and phospholipids has also been described for other species of the genus *Microlunatus* (Kämpfer *et al.*, 2010).

Fatty acid analysis was performed according to Kämpfer & Kroppenstedt (1996), except that strains were cultivated on R2A agar for 5 days at 28 °C prior to analysis.

The fatty acid profile of strain 12-Be-011^T was similar to those of the other closely related species *M. phosphovorus*, *M. ginsengisoli* and *M. panaciterrae* with predominant amounts of iso-$C_{15:0}$ (between 17 and 46 %) and anteiso-$C_{15:0}$ (between 32 % and 53 %), but minor differences (no traces of saturated and unsaturated fatty acids) could also be observed (see Supplementary Table S1 in IJSEM Online). The results of the comparative physiological characterization are given in Table 1 and the species description, with methods as described previously (Kämpfer *et al.*, 1991). DNA–DNA hybridization experiments were not performed between strain 12-Be-011^T and the type strains of the genus *Microlunatus* because of the low 16S rRNA gene sequence similarities (<97 %) to all other species of this genus. The differences observed in the fatty acid profiles and in the results of the physiological tests between the type strains of species of the genus *Microlunatus* (Table 1 and Supplementary Table S1) clearly warrant the creation of a separate species.

Table 1. Physiological characteristics of the type strains of the genus *Microlunatus*

Strains: 1, 12-Be-011^T; 2, *M. soli* CC-12602^T; 3, *M. aurantiacus* DSM 18424^T; 4, *M. phosphovorus* DSM 10555^T; 5, *M. ginsengisoli* DSM 17942^T; 6, *M. panaciterrae* Gsoil 954^T. Data for *M. panaciterrae* are from An *et al.* (2008); all other data are from this study. All strains were positive for utilization of: L-arabinose*, D-glucose*, D-mannose*, maltose, melibiose, L-rhamnose, D-ribose and D-sorbitol. All strains were negative for the utilization of: D-gluconate, adipate, itaconate, L-alanine, 3-hydroxybenzoate and phenylacetate. +, Positive; −, negative; (+), weakly positive.

Characteristic	1	2	3	4	5	6
Assimilation of:						
Acetate	−	+	(+)*	−	(+)	−
N-Acetyl-D-glucosamine	(+)	+	+	+	+	−
Adonitol	+	+	−	+	+	−
p-Arbutin	−	+	−	+	+	−
Cellobiose	+	+	+	+	+	−
D-Fructose	+	+	+	+	+	−
D-Galactose	+	+	(+)*	+*	+*	−
L-Histidine	−	(+)	−	−	(+)	−
i-Inositol	+	+	+*	+	+	−
L-Malate	−	(+)	(+)*	+	+	−
Maltitol	+	+	+	+	+	−
D-Mannitol	+	+	+*	+	+	−
L-Proline	−	(+)	−	−	(+)	−
Propionate	−	+	−*	−	−	−
Salicin	−	+	−	+	+	+
L-Serine	−	(+)	−*	−	−	−
Sucrose	+	+	+	−	+	+
Trehalose	+	+	+	+	+	−
D-Xylose	+	+	+*	+*	+*	−

*Data are in agreement with those of An *et al.* (2008).

Description of *Microlunatus parietis* sp. nov.

Microlunatus parietis (pa.ri′e.tis. L. gen. n. *parietis* of the wall of a house).

Rod-shaped and coccoid cells, Gram-positive, oxidase-positive, showing an aerobic respiratory metabolism. Good growth occurs after 5 days incubation on R2A agar, tryptone soy agar, M79 agar and nutrient agar at 25–30 °C. The peptidoglycan type is A3γ based on LL-2,6-diaminopimelic acid. The quinone system comprises menaquinones MK-9(H_4) (58 %), MK-8(H_2) (28 %) and minor amounts of MK-8 (10 %) and MK-9 (3 %). The polar lipid profile consists of diphosphatidylglycerol, phosphatidylglycerol, phosphatidylinositol, two unknown phospholipids and one unknown glycolipid. Major fatty acids are iso-$C_{15:0}$, anteiso-$C_{15:0}$ and, iso-$C_{16:0}$ (see Supplementary Table S1 in IJSEM Online). Many carbon sources are utilized, including *N*-acetyl-D-glucosamine, L-arabinose, cellobiose, D-fructose, D-galactose, D-glucose, D-mannose, maltose, melibiose, L-rhamnose, D-ribose, sucrose, trehalose, D-xylose, adonitol, i-inositol, maltitol, D-mannitol and D-sorbitol. p-Arbutin, D-gluconate, acetate, adipate, *cis*-aconitate, citrate, propionate, L-malate, mesaconate, L-alanine, L-histidine, L-proline, L-serine, 3-hydroxybenzoate and phenylacetate are not utilized (Table 1).

The type strain, 12-Be-011^T (=DSM 22083^T=CCM 7636^T), was isolated by C. Trautmann from an indoor wall in Berlin, Germany.

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